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EFFECTS OF SIMULATED SPACE ENVIRONMENTS
ON THE VIABILITY OF MICROORGANISMS

Quarterly Status Report
October 16, 1962 through January 15, 1963

by

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INTRODUCTION

This report under Contract No. NASr-41, "Effects of Simulated Space Environments on the Viability of Microorganisms", National Aeronautics and Space Administration, Washington, D. C., summarizes the results obtained during the period October 16, 1962 through January 15, 1963. The experimental program was a joint effort of National Research Corporation and the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts. The previous report was dated November 2, 1962 and described the work accomplished during the period July 16 through October 15, 1962.

The present report includes a discussion of the survival of organisms from soils exposed to ultrahigh vacuum at temperatures of 120 and 150°C. The resistance of organisms isolated from several desert soils subjected to vacuum and 120°C was investigated by placing the cultured organisms in vacuum at 120°C. Experiments have been initiated in which spores of 5 organisms are irradiated with ultraviolet light while under ultrahigh vacuum.

A new vacuum chamber heating system has been fabricated which will reach isothermal temperatures to 200°C at lower pressures than had been possible in previous work. This system was designed to provide the resistance wire heat source within the chamber rather than externally as in the past. Water-cooling coils were also added to the exterior of the vacuum chamber walls to maintain low wall temperature and minimize system outgassing. This equipment was used in the temperature-vacuum studies described here.

Gamma radiation experiments on organisms sealed in vacuum will be conducted in a new apparatus. This system was constructed with ultrahigh vacuum valves as integral components of the tubulation system. It will be possible to determine pressures within the individual tubes at any time until the organisms are plated.

A paper entitled "Combined Effects of Ultrahigh Vacuum and Temperature on the Viability of Some Spores and Soil Organisms", by Davis, Silverman, and Keller, has been accepted for publication in Applied Microbiology.

TEMPERATURE EXPERIMENTS

Desert Soil

Three Mohave Desert soils were placed in ultrahigh vacuum at 120 and 150°C for 4.5 days and then plated by procedures described in previous reports. Heterotrophic mesophilic and thermophilic bacteria, molds, and actinomycetes were determined. Cultures were incubated aerobically and anaerobically. Although the plating technique was designed to optimize recovery of organisms, few colonies appeared on each plate showing growth. It was necessary to confirm the resistance of the isolates as well as rule out chance contaminants by returning the isolates to the vacuum chamber at high temperature. These cultures are also being placed at elevated temperatures at atmospheric pressure.

Fifty of the aerobic organisms recovered from experiments at 120°C in ultrahigh vacuum were returned to the vacuum chamber at that temperature for 4.5 days. A qualitative screening procedure was devised to determine survival. Reeve Angel 2.1 cm No. 934-AH glass fiber filter paper circles were numbered in duplicate by a color code system and sterilized by autoclaving. The growth on agar slants of the organisms picked from soil plates was scraped and spread on moistened filters. These tryptone glucose extract agar (TGE) slants were the initial subcultures taken from the soil plates and were several weeks old. The prepared filters were then dried at 45°C for 2.5 hr and stored overnight at 20°C in a silica gel desiccator. One filter of each organism was then plated to determine whether the drying process was lethal, while the other filter was subjected to ultrahigh vacuum and 120°C for 4.5 days.

Both sets of filters were plated by impressing the filter surface bearing the organisms on fresh TGE plates at 5 positions around the plate periphery. The filter was then impressed in the center of the plate and left on the plate. Cultures were incubated at 30°C and examined daily for growth. The experiment was concluded after plates had incubated for 12 days. Plates from filters which had been in vacuum at 120°C and showed growth were picked, and colonies transferred to TGE slants. All plates were then flooded with 0.2% triphenyl tetrazolium chloride. The identity of cultures recovered from the vacuum chamber with the original isolates was confirmed by comparing the cultural characteristics and by microscopic examination.

Only one of the 50 cultures did not survive the moderate drying treatment used to prepare the samples. Within the 12 days incubation period, 8 of the 50 cultures which had been in the vacuum chamber at 120°C showed growth conforming to the original isolates. Seven cultures were colorless, punctiform, and sporeforming, while the other culture was amber and butyrous. These cultures will be identified to species if possible.

ULTRAVIOLET EXPERIMENTS

Experiments have been initiated which will compare the viability of five test spores after ultraviolet (UV) irradiation in ultrahigh vacuum and at atmospheric pressure. These experiments are being conducted over a wide range of UV doses.

Known numbers of spores (10^2 - 10^6) on glass fiber filter paper circles and membrane filters at the same test conditions are used to compare survival data when spores are shielded from UV within the glass fiber mat and spores are almost entirely exposed to UV on the membrane filter surface. Any reduction in population due to vacuum is being monitored by shielding a similar group of filters from UV but exposing them to vacuum.

The influence of moisture on spore viability after UV exposure is also being studied. A group of filters from each UV-vacuum experiment is placed in a desiccator containing water and stored at 20°C for a day before they are processed. Filters are normally

plated the day they are removed from the vacuum chamber. Undried filters are to be irradiated at atmospheric pressure. Dried filters stored in a silica gel desiccator for the duration of the 5-day stay in vacuum serve as an additional control. No effort is being made to shield spores from visible light. The spores include Bacillus stearothermophilus, B. megaterium, B. subtilis var. niger, Clostridium sporogenes, and Aspergillus niger.

A newly designed stainless steel framework was constructed to support the filters and the ultraviolet lamp within the vacuum chamber. The lamp was mounted centrally so that filters faced the lamp at a distance of 10 cm from the lamp wall. The same apparatus was used to irradiate filters at atmospheric pressure.

Experiments performed so far used 30 sec and 10 min UV after 5 days in ultrahigh vacuum. These irradiation periods corresponded to doses of 15,000 and 200,000 ergs respectively. A thermopile assembly sensitive to wavelengths below 3000 \AA was used to measure the UV radiation incident on a blank set of filters mounted on the framework. The dosimetry will be repeated with a germicidal UV intensity meter which responds only to energy at 2537 \AA .

RESULTS AND DISCUSSION

When membrane filters holding 10^6 spores were given 15,000 ergs UV in vacuum and at atmospheric pressure, 0.01 - 0.1% of the spores were recovered. Some differences in survival in vacuum and at atmos-

pheric pressure were observed, but these were not significant because of the small number of viable spores. The results with glass filters on the other hand demonstrated that spores within the fiber mat did not receive much if any UV since 0.1 - 50% of the spores were viable. B. subtilis var. niger was the only organism for which less than 20% viability was noted.

At 200,000 ergs, about 0.001% of the spores on membrane filters were recovered with the exception of A. niger which gave about 0.1% survival. On glass filters, 0.01% of the B. subtilis var. niger spores, 0.5% of the A. niger spores, and 10% of the three other spores were viable when compared with specimens exposed to vacuum but shielded from UV.

The data obtained from filters stored over water before they were plated did not correlate well in two experiments, but these observations must be viewed in the context of a complete series of experiments before conclusions may be drawn. At 200,000 ergs, 50-1000% of the irradiated organisms plated the day they were removed from the vacuum chamber were recovered from filters stored over water. A. niger gave the apparent ten-fold increase in recovery in this experiment, but filters of this organism given 15,000 ergs were unchanged in stability.

Improvements in the UV lamp system will be made to provide better dose control at low flux levels. Low dosages are currently obtained by energizing the lamp for short periods of time, (e.g. 30 seconds). The lamp does not reach a steady output level for several minutes and therefore the exact value and repeatability of

low dosages is subject to variance. A new power system will be constructed to control the lamp internally so that low fluxes can be studied without resorting to short exposure periods.